BRIEF REPORT

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Rapamycin selectively alters serum chemistry in diabetic mice

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The study was undertaken to explore the effect of rapamycin, an anti-inflammatory agent, on the metabolic profile of type 2 diabetic mice. Seven-month-old diabetic db/db mice and their lean littermate non-diabetic controls (db/m) were randomized to receive control chow or chow mixed with rapamycin (2.24 mg/kg/day) (each group $n=20$, males and females) for 4 months and sacrificed. Serum samples were analyzed for the measurement of glucose, creatinine, blood urea nitrogen (BUN), alkaline phosphatase (ALP), alanine aminotransferase (ALT), total cholesterol, total triglyceride, and total protein, using the automated dry chemistry analysis. Rapamycin elevated serum glucose in female diabetic mice. Serum creatinine tended to be higher in diabetic mice but was not affected by rapamycin; there was no difference in BUN levels among the groups. Serum ALP was elevated in diabetic mice and rapamycin lowered it only in female diabetic mice; serum ALT levels were increased in female diabetic mice, unaffected by rapamycin. Serum total protein was elevated in diabetic mice of both genders but was not affected by rapamycin. Diabetic mice from both genders had elevated serum cholesterol and triglycerides; rapamycin did not affect serum cholesterol but decreased serum total triglycerides in male diabetic mice. We conclude that rapamycin elicits complex metabolic responses in aging diabetic mice, worsening hyperglycemia in females but improving ALP in female diabetic and total triglycerides in male diabetic mice, respectively. The metabolic effects of rapamycin should be considered while performing studies with rapamycin in mice.

Keywords: *alkaline phosphatase*; *alanine aminotransferase*; *cholesterol*; *triglycerides*

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The prevalence of diabetes is steadily increasing in the United States; 1.3 million new cases are diagnosed each year in the United States with 90–95% accounting for type 2 diabetes (1). Chronic complications of diabetes tend to be more severe among older adults (2). Rapamycin, an anti-inflammatory agent used to prevent organ transplant rejection, extends life span in mice (3). Rapamycin is known to increase lipids in humans (4,5). Since many earlier investigations with rapamycin, including our own, employed young rodents (6), its effect on metabolic profile in older mice with or without diabetes is not known. It is very likely that rapamycin will be employed in future studies in aging mice with or without diabetes. Thus, our aim was to assess metabolic risks with rapamycin in mice with or without diabetes.

Materials and methods

Animals

We employed C57BLKsJ lepr −/− db/db mice with type 2 diabetes and their non-diabetic db/m littermates in our study (Jackson Laboratory, ME) (each group $n=20$, males and females). The diabetic mice succumb within 12 months of age (7), suggesting they have a short life span. Accordingly, experiments were initiated at 7 months of age when diabetic mice were hyperglycemic for at least 5 months. The db/m and db/db mice were each divided

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Table 1. Effect of rapamycin on body weight and select serum chemistry in non-diabetic (db/m) and diabetic (db/db) male mice

	db/m–control diet	db/m–rapa diet	db/db–control diet	db/db–rapa diet
Body weight (g)	37.22 ± 1.2	37.35 ± 1.5	30.76 ± 3.4*	26.81 ± 1.6
Glucose (mg/dl)	86.06 ± 4.7	80.25 ± 4.1	446.1 ± 47.2***	442.2 ± 40.3
Creatinine (mg/dl)	0.44 ± 0.03	0.36 ± 0.02	0.51 ± 0.04	0.48 ± 0.05
BUN (mg/dl)	20.37 ± 1.2	21.16 ± 0.9	23.08 ± 2.1	22.91 ± 2.6
ALP (IU/L)	40.63 ± 2	45.95 ± 1.4	88.43 ± 8.7***	92.09 ± 8.3
ALT (IU/L)	27.37 ± 3	35.21 ± 5	43.00 ± 5	42.00 ± 2
Total protein (g/dl)	4.81 ± 0.08	4.85 ± 0.06	5.60 ± 0.1***	5.35 ± 0.2
Total cholesterol (mg/dl)	71.74 ± 3.2	70.32 ± 2.6	111.9 ± 10.5***	96.91 ± 5.5
Total triglycerides (mg/dl)	103.5 ± 4.6	92.6 ± 2.5	139.3 ± 13***	89.9 ± 8.6†††

*$p < 0.05$ vs. dbm-control diet, ***$p < 0.001$ vs. dbm-control diet, †††$p < 0.001$ vs. db/db mice on control diet.

into two groups at 7 months of age, and one group received laboratory chow with microencapsulated rapamycin whereas the other group received chow with empty capsules as described (3). All four groups were followed up for a period of 4 months on *ad lib* feeding and sacrificed at 11 months. At the time of sacrifice, blood was collected from the periorbital area after anesthesia. Serum was prepared by spinning the tubes of fresh blood prior to metabolic profile analysis. These experiments were approved by our Institutional Animal Care and Use Committee.

Automated dry chemistry analysis

Analysis of the following metabolic biomarkers in serum was performed using the Automated Dry Chemistry Analyzer machine Spotchem EZ SP-4430: glucose, total protein, creatinine, blood urea nitrogen (BUN), alkaline phosphatase (ALP), alanine aminotransferase (ALT), total cholesterol and total triglyceride.

Statistical analysis

Data were expressed as means and standard error; statistical comparison between multiple groups of males and females mice was performed by analysis of variance with Newman-Keuls correction. A p value of <0.05 was considered significant.

Results and discussion

At the age of 7 months, the male db/db mice on control diet weighed less than the non-diabetic males on a similar diet ($p < 0.05$); in contrast, the female diabetic mice on control diet were heavier than non-diabetic females on the same diet ($p < 0.05$). Rapamycin did not affect body weights in non-diabetic or diabetic mice of either gender (Tables 1 and 2). The effect of rapamycin on metabolic parameters was separately analyzed for males and females because gender is an important determinant of aging-associated target organ damage (3,8). As expected, the serum glucose levels were elevated in male and female diabetic mice compared to the respective non-diabetic mice ($p < 0.001$) (Tables 1 and 2). Rapamycin did not affect the ambient serum glucose levels in non-diabetic or diabetic male mice, in agreement with studies in mice following pancreatic islet transplantation (9). However, rapamycin induced worsening of hyperglycemia in female diabetic mice (Table 2). Female diabetic mice tended to have decreased level of serum glucose compared to

Table 2. Effect of rapamycin on body weight and select serum chemistry in non-diabetic (db/m) and diabetic (db/db) female mice

	db/m–control diet	db/m–rapa diet	db/db–control diet	db/db–rapa diet
Body weight (g)	31.51 ± 1.2	28.06 ± 0.6	38.94 ± 3.2*	36.04 ± 2.7
Glucose (mg/dl)	76.61 ± 13.7	45.39 ± 2.7	348.0 ± 38.2***	438.0 ± 37.4†
Creatinine (mg/dl)	0.37 ± 0.02	0.38 ± 0.02	0.46 ± 0.05	0.49 ± 0.03
BUN (mg/dl)	19.88 ± 1.2	19.71 ± 1.5	21.56 ± 1.2	24.94 ± 3.4
ALP (IU/L)	73.88 ± 3.3	87.47 ± 3.7	126.7 ± 9.5***	106.6 ± 5.5†
ALT (IU/L)	28.12 ± 3.9	32.41 ± 1.6	76.63 ± 13.7***	62.75 ± 9.3
Total protein (g/dl)	4.47 ± 0.1	4.38 ± 0.1	5.56 ± 0.1***	5.33 ± 0.1
Total cholesterol (mg/dl)	51.82 ± 1.8	50.00	113.3 ± 13.4***	110.1 ± 7.7
Total triglycerides (mg/dl)	94.82 ± 6.9	83.76 ± 3.2	131.3 ± 14.4*	113.8 ± 10.6

*$p < 0.05$ vs. dbm-control diet, ***$p < 0.001$ vs. dbm-control diet, †$p < 0.05$ vs. db/db mice on control diet.

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diabetic male mice but this did not reach statistical significance (Table 2).

Serum creatinine and BUN are commonly used as indicators of glomerular filtration rate of the kidney. There was a non-significant trend toward increase in serum creatinine concentration in diabetic mice compared to non-diabetic mice (Tables 1 and 2); this was unaffected by rapamycin. Perhaps because of more muscle mass in males, serum creatinine levels tended to be higher in male non-diabetic and diabetic mice compared to their respective female counterparts. BUN levels were unchanged in diabetic male and female mice compared to the respective non-diabetic mice (Tables 1 and 2). Rapamycin did not alter BUN levels significantly in either the male or the female non-diabetic or diabetic mice. There are conflicting data on the effect of rapamycin on renal function in humans. In 19 type 1 diabetic patients who received rapamycin, serum creatinine was mostly unchanged (10). Taken together, these data suggest the context of kidney injury may determine whether rapamycin causes nephrotoxicity.

Effect of rapamycin on liver function was evaluated in control and diabetic mice. The serum ALP level was significantly elevated in diabetic mice compared to non-diabetic mice regardless of gender ($p < 0.001$) (Tables 1 and 2). Rapamycin did not affect the serum ALP level in male non-diabetic or diabetic mice (Table 1). However, rapamycin significantly lowered the parameter in female diabetic mice compared to those treated without rapamycin ($p < 0.05$, Table 2). The serum ALT level tended to be higher in male diabetic mice (Table 1). The female diabetic mice exhibited a significantly higher level of ALT compared to female non-diabetic mice ($p < 0.001$, Table 2). ALT levels were not affected by rapamycin in either diabetic or non-diabetic mice of either gender. The increase in ALT in diabetic mice may be consistent with nonalcoholic fatty liver disease, which is common in obesity and type 2 diabetes (11). Since rapamycin inhibits the activity of mTOR complex1 and it induced improvement in serum ALP in female diabetic mice in our study, more investigation is needed to establish whether mTOR complex1 is activated in the liver in obesity and type 2 diabetes.

Serum total protein level was elevated in male and female diabetic mice compared to the respective non-diabetic mice ($p < 0.001$, Tables 1 and 2). Rapamycin administration did not affect the serum protein levels in diabetic or non-diabetic mice of either gender.

Elevated serum cholesterol and triglycerides are linked to atherosclerosis. Several studies have shown association between rapamycin and increased serum triglyceride and cholesterol levels in human subjects (4,5). Diabetic mice of both genders had higher serum cholesterol ($p < 0.001$) and higher triglycerides ($p < 0.001$ for males, $p < 0.05$ for females) relative to non-diabetic mice (Tables 1 and 2). Surprisingly, we did not observe worsening of hypercholesterolemia or hypertriglyceridemia with rapamycin in either the diabetic mice or the non-diabetic mice in contrast to previous reports (12). In fact, rapamycin exerted a significant beneficial effect on serum triglycerides in diabetic male mice (Table 1). Differences in species and genetic background could account for the lack of effect of rapamycin on serum lipids in mice used in our study. We also noted differences in the effect of rapamycin between males and females, e.g. ALP and total triglycerides. It is possible that the differences in hormonal profile between males and females could vary the response of metabolic pathways to rapamycin. It should be noted that, in the study by Harrison et al. (3), the effect of feeding rapamycin on lifespan was greater in female mice than in male mice. Gender difference in responses to mTOR inhibition was also reported by Selman et al.; thus, female p70S6 kinase1 knockout mice had significantly longer lifespan than their male counterparts (13). In conclusion, our studies suggest that rapamycin has complex metabolic effects in diabetic mice. In comparison with previous reports, our studies suggest that metabolic effects of rapamycin can vary depending on the context such as species, gender, and diabetes.

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Conflict of interest and funding

The authors have not received any funding or benefits from industry or elsewhere to conduct this study.

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